Cell & Gene Therapy Ltd.

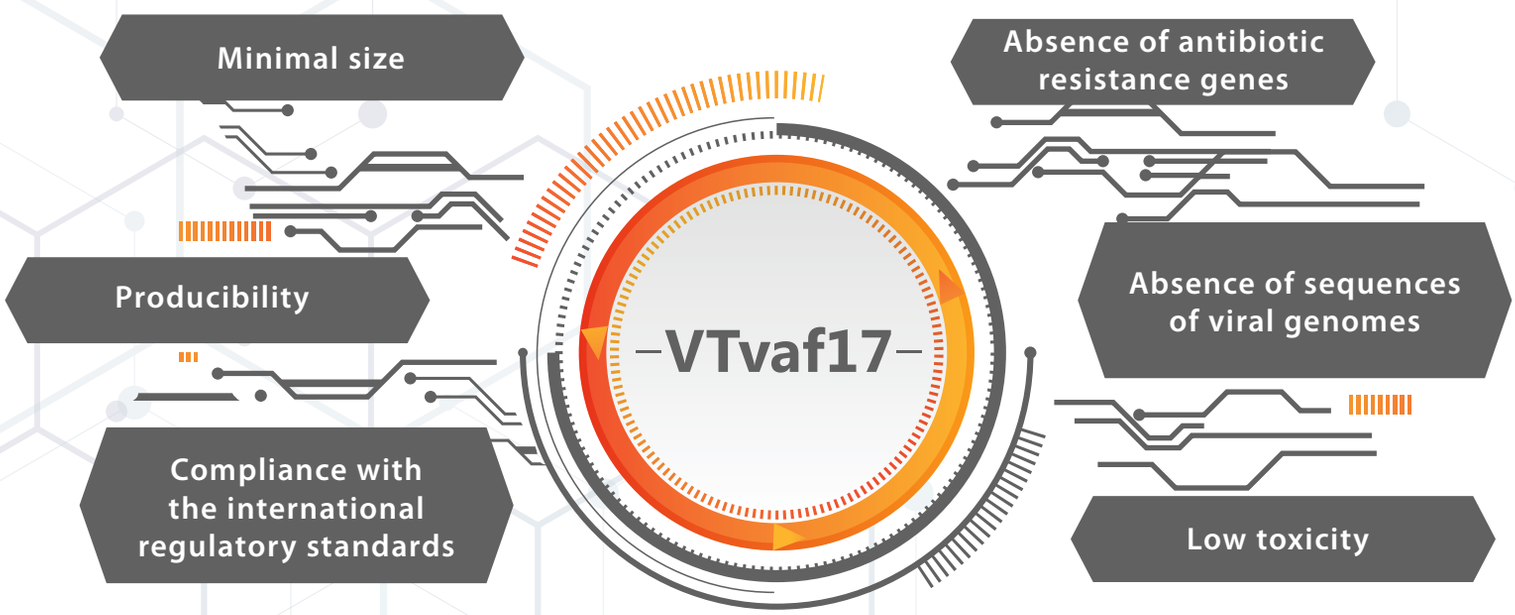
EFFECTIVE AND SAFE DNA-VECTOR OF VTvaf17 SERIES FOR CELL AND GENE THERAPY

EFFECTIVE AND SAFE DNA-VECTOR OF VTvaf17 SERIES FOR CELL AND GENE THERAPY

Gene therapy is a treatment of hereditary and multifactorial diseases by introducing genes in the patients' cells or correcting of own genes properties with the aim of a controlled modification of gene defects, compensating for the work of the damaged genes or assigning new functions to the cells.

Viral and nonviral vectors are used as carriers of therapeutic genes. Plasmids are used as nonviral vectors most of the time.

The developed VTvaf17 vector is a DNA-vector of the latest generation, combining the effectiveness of viral and the safety of plasmid vectors.



Minimal size

It is known that the longer the length of a plasmid vector the less effectively it penetrates into the target cell. The existing plasmid vectors are often overloaded by non-functional regions, which increase the vector size significantly. Besides, in case of the cells transfection by plasmid vectors, toxicity is often observed.

The size of gene therapy DNA-vector VTvaf17 is only 3165 b.p., enabling to effectively transfect cells of various tissues without toxic effect.

Safety for patients – absence of elements of viral genomes

A significant limitation of the use of the existing therapeutic plasmid vectors is the presence of regulatory elements for the increase of target genes expression (promoters, enhancers, post-translational regulatory elements), which, in most cases, represent nucleotide sequences of the genomes of various viruses.

European Medicines Agency recommends to avoid including such sequences in the therapeutic plasmid vectors (Draft Guideline on the quality, non-clinical and clinical aspects of gene therapy medicinal products, EMA/CAT/80183/2014, Committee for Advanced Therapies).

Gene therapy DNA-vector VTvaf17 does not contain the sequence of viral genomes. The expression of therapeutic genes is realized by the promoter region of human elongation factor EF1a gene with own enhancer contained in the first intron of the gene. It ensures a high level of transcription of the therapeutic gene in the majority of human tissues.

Epidemiological safety – absence of antibiotic resistance genes

The existing therapeutic plasmid vectors contain resistance genes to various antibiotics for selection of the transformed bacterial strains. It is with the help of plasmid vectors that the mechanism of horizontal gene transfer, including antibiotic resistance genes, within the population of microorganisms, which gives them with a selective advantage. Thus, the horizontal gene transfer is associated with the spread of pathogens of human infectious diseases resistant to modern antibiotics.

The European Medicines Agency considers it necessary to avoid introducing antibiotic resistance markers into the developed plasmid vectors for gene therapy (Reflection paper on design modifications of gene therapy medicinal products during development /14 December 2011 EMA/CAT/GTWP/44236/2009 Committee for advanced therapies).

Gene therapy DNA-vector VTvaf17 does not contain antibiotic resistance genes. The selection of the transformed strains is ensured by the complex RNA-out, which represents a short antisense RNA. Sucrose acts as a selective agent. Gene therapy DNA-vector VTvaf17 may be amplified only in a specially developed by us strain *Escherichia coli* SCS110-AF and cannot transform other bacterial strains, including those that enter in the composition of a human microbiota.

Low toxicity

Gene therapy DNA-vectors VTvaf17 (with encoding genes insertions) demonstrated extremely low toxicity at the stage of preclinical studies:

- based on the results of acute toxicity studies the drug was classified as the 6th class of toxicity, i.e. relatively non-hazardous substances;
- based on the results of chronic toxicity tests on rabbits and rats the drug was ranked as the III-rd hazard class, i.e. low-hazard class;
- based on the results of chronic toxicity tests on female rhesus macaques there were no deaths of animals, no clinical evidence connected with toxic effect of the studied drug have been observed;
- gonadotoxic, allergizing, immunotoxic and mutagenic effects – have not been found.

Compliance with the international regulatory standards

DNA-vector VTvaf17 meets the regulators' requirements for genetic medicinal products: Draft Guideline on the quality, non-clinical and clinical aspects of gene therapy medicinal products, (EMA/CAT/80183/2014, Committee for Advanced Therapies), Reflection paper on design modifications of gene therapy medicinal products during development (14 December 2011 EMA/CAT/GTWP/44236/2009 Committee for advanced therapies).

The product was developed in strict accordance with guidance recommendations FDA-2014-D-0663 concerning determining the need for and content of environmental assessments for gene therapies, vectored vaccines, and related recombinant viral or microbial products.

The production and quality control, including the safety indicators (apyrogenicity, sterility, stability, purity, biological activity etc.), of the developed vectors are performed in accordance with the requirements of U.S. Pharmacopeia: USP 42 and NF 37 chapter 1047 (Gene Therapy Products), and, also, according to guidance recommendations FDA-2008-D-0205 "Chemistry, Manufacturing, and Control (CMC) Information for Human Gene Therapy Investigational New Drug Applications (INDs) Guidance for Industry" as well as FDA-2015-D-3399 "Recommendations for Microbial Vectors used for Gene Therapy".

Producibility features

A certain advantage of plasmid vectors over the viral ones is the simplicity and lower costs of plasmid DNA development. Nevertheless, the output of the target product, at the moment, is not more than 10 mg of plasmid DNA per 1 litre of bacterial culture, which is not sufficient for commercial production of gene therapy drugs on the base of plasmid DNA. In addition, because of the bacterial lipopolysaccharides that make up the E.coli cell wall, the process of obtaining plasmid DNA suitable for use as a medicine is complicated and costly.

We have developed a process flow scheme for production and purification of gene therapy DNA-vector VTvaf17 with the output of more than 200 mg of the target product from 1 litre of the bacterial culture that meets the strictest contemporary requirements to purity of the developed gene therapy drugs on the base of nucleic acids.

Field of application

The use of currently existing therapeutic plasmid vectors is limited due to the above mentioned reasons. The new innovative DNA-vector VTvaf17 is the first modern effective and safe tool with a wide potential of use in various fields of medicine:

- Genetic therapy of hereditary diseases compensating for the function of the defective gene;
- Genetic therapy of multifactorial diseases to increase the target genes expression;
- Use as a safe vector for genomic engineering, including, genome editing;
- cell therapy of oncological diseases, including the use as the main effective and safe vector for CAR-T therapy in oncohaematology;
- immunotherapy, including the development of therapeutic antibodies in vivo and DNA-vaccination.

The developed gene therapy DNA-vector VTvaf17 is innovative and has undeniable advantages over the already existing plasmid vectors:

	Existing plasmid vectors	Gene therapy vectors of VTvaf17 series
Plasmid part size	> 3 000 b.p.	< 2 000 b.p.
Toxicity during the transfection	Yes	No
Antibiotic resistance gene	Yes	No
Selective agent	Antibiotic	Sucrose
Selective marker type	Protein	RNA
Target product output	< 10 mg/litre	> 200 mg/litre
Scalability of production	No	Yes
Transgene tissue-specific expression	Yes/No	No
Safety for the environment	Capable of replication in various pUC-compatible strains	Capable of replication only in a special strain

Patents

Patent: RU 2 678 756, International Application Number: PCT/RU2018/000191, WO2019/039962, USA Application Number: 16636713; Title of Invention: "Gene therapy DNA-vector VTvaf17, production method, strain *Escherichia coli* SCS110-AF, production method, strain *Escherichia coli* SCS110-AF/ VTvaf17, bearing gene therapy DNA-vector VTvaf17, production method". Date of invention: 25.08.2017.

CELL and GENE Therapy Ltd,
Reg#: 9415326,
Address: 41 Devonshire street, LONDON,
W1G 7AJ, UK
E-mail: info@cgtgb.com
Tel: +44 7349 056444